Last date revised: August 24, 2011

Cryopreservation of Chicken Gonads on Acupuncture Needles

PURPOSE:
The cryopreservation of chicken gonads procedure is used to maintain genetic lines through preservation of ovaries and testes.

POLICY:
Removal of ovaries and testes for cryopreservation in chicken should optimally be performed within 24 hours of hatch. In chicks older than 1 day of age the transplantation of gonadal tissue becomes entirely dependent on the use of an immunosuppressant.

RESPONSIBILITY:
Technician, veterinarian, research associate

MATERIALS:
Ethylene Glycol (EG)
Dimethylsulfoxide (DMSO)
Dulbecco's phosphate buffered saline (DPBS)
Fetal Bovine Serum (FBS)
Sucrose
Sterilized sharp surgical scissors, scalpel blade, fine forceps (Dumont),
Sterile gauze
Petri dish
Ice
Liquid nitrogen
Styrofoam box
50 ml tubes
10 ml tubes
Dissecting microscope

All methods used were approved by the Pacific Agri-Food Research Center (Agassiz) Animal Care Committee and followed principles outlined by the Canadian Council of Animal Care (reformatted 2011).
http://www.ccac.ca/en_/standards/guidelines

PROCEDURE:

1. Newly hatched chicks are obtained from exterior sources on by incubation and hatching on premises. Weak or damaged chicks are culled by cervical dislocation, and healthy chicks are transferred to a warm brooder box (37.1 °C) in the lab.

2. Media is prepared:
   a. For holding tissues until vitrification: Dulbecco's phosphate buffered saline (DPBS) with 20% Fetal Bovine Serum (FBS).
   b. Equilibrium solution: 7.5 % EG + 7.5 % DMSO + DPBS with 20% FBS.
   c. Vitrification solution: 15% EG + 15% DMSO + 0.5 M sucrose + DPBS with 20% FBS.

3. Chicks are killed by cervical dislocation and the abdominal cavity is opened to expose the gonads.

4. In chicks, the testes are paired organs located in the dorsal part of the abdomen on either side of the median plane. The single ovary is an irregularly shaped structure attached to the dorsal abdominal wall on the left side of the cavity.

5. Gonads are detached with fine forceps and/or fine scissors. Usually only the cortical part of the ovary, which contains the germinal cells, is removed.

6. With the aid of a dissecting microscope, connective tissue surrounding and under the gonads is removed.

7. The end of each testicle is cut to allow the media (cryoprotective agent) to penetrate the tissue. Right testicles are placed in one dish and left testicles are placed in a second dish to ensure that individuals will not be represented more than once when material is recovered.

8. Gonads are kept on ice in approximately 5.0 mL of media (DPBS + 20% FBS) from the time that they are removed, and are cryopreserved within 4 h.

9. After all tissue is collected, the ovaries are cut in half and the two halves of each ovary are put on one acupuncture needle. A second ovary cut in half is then put on the same needle. Testes are placed whole on an acupuncture needle with two testes per needle.

10. The needles holding gonads are placed in the equilibration solution for 10 minutes.

11. The needles are transferred to a vitrification solution for 2 minutes.

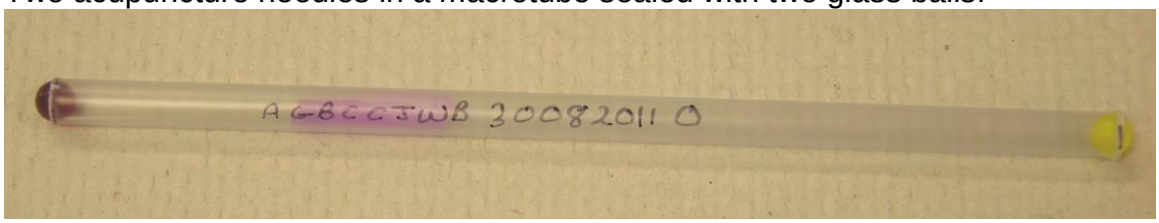
12. The gonadal tissue is blotted on gauze to remove excess solution and immediately plunged into LN_2. This is done wearing gloves and working in a styrofoam cooler with LN_2 at a level just below a mini

metal work bench in the cooler. After the samples have stopped boiling in the liquid nitrogen, two needles (two ovaries or two testicles) are placed in a 2 ml pre-cooled macrotube with one end previously sealed with a glass ball. The other end of the macrotube is then sealed with a glass ball while working in the LN_2 vapour on the mini metal work bench.

The macrotubes are temporarily stored in the LN_2 in the styrofoam cooler. When a group of macrotubes are sealed the tubes are transferred to the LN_2 storage tank

Ovaries on acupuncture needles.

Two acupuncture needles in a macrotube sealed with two glass balls.



Styrofoam cooler with mini metal work bench. LN_2 is added at a level just below the bench.